PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/66

A1

(11) International Publication Number: WO 99/60406

(43) International Publication Date: 25 November 1999 (25.11.99)

(21) International Application Number: PCT/US99/11201

(22) International Filing Date: 21 May 1999 (21.05.99)

(30) Priority Data:
09/082,542 21 May 1998 (21.05.98) US

(71) Applicant: INSMED PHARMACEUTICALS, INC. [US/US]; 800 E. Leigh Street, Richmond, VA 23229 (US).

(72) Inventors: ALLAN, Geoffrey; 105 Westham Parkway, Richmond, VA 23229 (US). OPRANDY, John, J.; 17914 Hickman Street, Poolesville, MD 20837 (US).

(74) Agents: FOX, Samuel, L. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005–3934 (US).

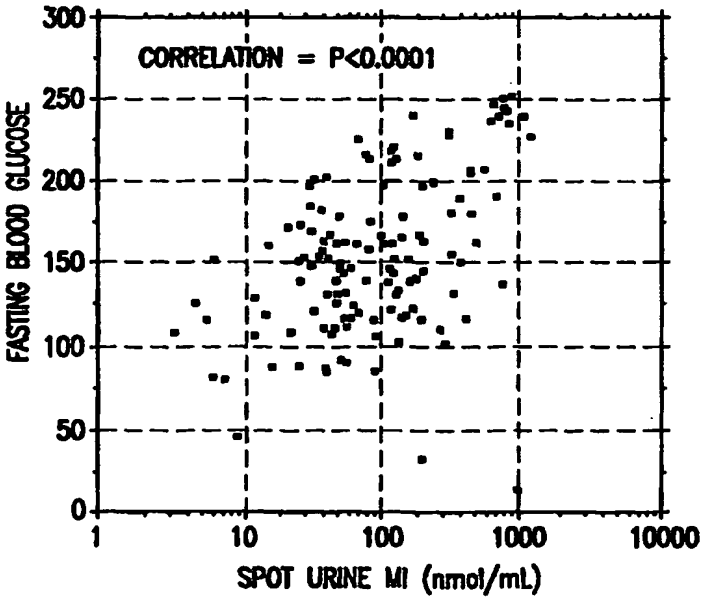
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: *MYO*–INOSITOL AS A PREDICTOR OF GLYCEMIC CONTROL IN MAMMALS



(57) Abstract

This invention relates to methods of assessing glycemic control in mammals with Type II diabetes by measuring the level of *myo*–inositol in body fluid.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

MYO-INOSITOL AS A PREDICTOR OF GLYCEMIC CONTROL IN MAMMALS

FIELD OF THE INVENTION

This invention relates to methods of assessing glycemic control in mammals with Type II diabetes by measuring the level of *myo*-inositol in body fluid.

BACKGROUND OF THE INVENTION

Myo-inositol (MI) is an isomer of inositol, a stable cyclic polyol. MI is found in blood, urine and most other tissues of the body. Sources of MI for the human body generally consist of food stuffs, but it is also produced in significant quantity by renal biosynthesis and is localized in quantity in the outer medulla of the kidney (Troyer *et al.*, *Annu. Rev. Physiol.*, *48*:51-71 (1986)).

Increased plasma and urine MI has been associated with glomerulonephritis and renal failure (Pitkanen, *Clin. Chim. Acta*, *20*:461-468 (1976); Melmed *et al.*, *Am. J. Med. Sci.*, *274*:55-59 (1977). Several mechanisms for explaining this observation have been proposed, including defects in tubular reabsorption and competition of glucose with the inositol transporter for renal uptake in the proximal tubule. The adverse effect of hyperglycemia on the uptake of MI via a Na+ dependent MI transporter in the proximal renal tubule has been demonstrated (Cole *et al.*, *Diabetes*, *44*:446-452 (1995)). A stereo specific MI/D-*chiro*-inositol transporter has been found in HepG2 cells (Ostlund *et al.*, *J. Biol. Chem.*, *271*:10073-10078 (1996)).

Significant amounts of MI in the urine patients with diabetes has long been observed (Pitkanen, *Clin. Chim. Acta*, *38*:221-230 (1972); Aloia, *J. Lab. Clin. Med.*, *82*:809-817 (1973)), perhaps even as early as 1858 (for review, *see* Hansen and Ortmeyer, *Lessons from Animal Diabetes VI*: 333-348 (1996)). These observations led early researchers to investigate the association of MI in urine to renal failure, as noted above. More recent reports have focused on the elevated excretion of MI in urine of patients with Type II diabetes (Kennington *et al.*, *N. Eng. J. Med.*, *323*:373-378 (1990), Ostlund *et al.*, *Proc. Natl. Acad. Sci. USA*, *90*:9988-9992 (1993);

Suzuki *et al.*, *Diabetes Care*, *17*:1465-1468 (1994)).

While the pattern of results seen in the various studies was similar, there was a wide variation in the absolute levels. For example, mean excretion of MI was reported by these various groups to be 91, 88 and 192 μmol/day, respectively, for normal individuals, and 270, 789 and 499 μmol/day, respectively, for patients with Type II diabetes (for review, *see* Larner and Craig, *Diabetes Care*, *19*:1-4 (1996)).

There is also evidence of increased excretion of MI in animals with Type II diabetes. Non-obese GK (Goto-Kazizaki) rats with Type II diabetes have been shown to manifest insulin resistance with increased urinary levels of MI (Suzuki *et al.*, in *New Directions in Research and Clinical Works for Obesity and Diabetes Mellitus*, Amsterdam, Elsevier, pp 197-203 (1991)).

Early detection and good glycemic control of Type II (non-insulin-dependent) diabetes is becoming increasingly recognized as important to reduce the development and progression of long term disease complications (The Diabetes Control and Complications Trial Research Group, *Diabetes*, *45*:1289-1298 (1996)). Glycemic control (*i.e.* the maintenance of normal or near-normal blood sugar levels) may involve diet, exercise, and weight reduction, as well as blood sugar monitoring and medication.

While there is evidence of increased excretion of MI in the urine of patients with Type II diabetes, to date MI levels have not been used to detect the degree of diabetes or glycemic control. Methods currently in use to assess the degree of glycemic control in Type II diabetes include the measurement of 1) fasting blood glucose followed by an oral glucose tolerance test (OGTT) and 2) plasma glycated hemoglobin and/or fructosamine. All of these tests require blood samples to be taken and the OGTT test necessitates an overnight fast followed by a morning attendance at a clinic for at least two hours. Accordingly, there remains a need for non-invasive and simpler methods to assess the degree of glycemic control in patients with Type II diabetes.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery that levels of MI in bodily fluids correlate to known and well accepted indicators of glycemic control in mammals with Type II diabetes. More specifically, it has now been discovered that urinary levels of MI correlate directly to three conventional indices of glycemic control: fasting blood sugar, fructosamine, and glycated hemoglobin.

Accordingly, a first embodiment of the present invention is directed to a method for assessing the degree of glycemic control in a mammal. This method comprises the steps of: (a) obtaining a sample of a bodily fluid from a mammal; (b) measuring the amount of MI in the sample; (c) comparing the amount of MI in the sample to previously defined reference amounts indicative of glycemic control; and (d) assessing the degree of glycemic control based on this comparison.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are intended to provide further explanation of the invention as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a graph showing the significant ($P<0.0001$) correlation between urinary MI levels and fasting blood glucose levels.

FIGURE 2 is a graph showing the significant ($P<0.0001$) correlation between urinary MI levels and blood fructosamine levels.

FIGURE 3 is a graph showing the significant ($P<0.0001$) correlation between urinary MI levels and blood glycated hemoglobin levels.

DETAILED DESCRIPTION OF THE INVENTION

Classically, Type II diabetes mellitus has been diagnosed by finding an elevated fasting blood glucose level. Ongoing assessment of the degree of glycemic control is done by measuring blood levels of fructosamine and/or glycated hemoglobin.

All of the tests currently employed require the collection of blood samples on a regular basis. There is therefore a need for a simple, non-invasive test for the assessment of glycemic control in mammals with Type II diabetes.

Accordingly, a first preferred embodiment of the present invention is directed to a method for assessing the degree of glycemic control in a mammal. This method comprises the steps of: (a) obtaining a sample of a bodily fluid from a mammal; (b) measuring the amount of MI in the sample; (c) comparing the amount of MI in the sample to previously defined reference levels indicative of glycemic control; and (d) assessing the degree of glycemic control based on this comparison.

To practice this first embodiment of the present invention, a sample of a bodily fluid must be obtained from the subject mammal. Preferably, the mammalian bodily fluid is blood or urine; more preferably, the mammalian bodily fluid is urine.

The sample of a bodily fluid may be taken from the mammalian subject without regard to the time of day or preceding fasting period. For example, the sample of a bodily fluid may be a "spot" sample (i.e. a sample taken at any time during the day, with or without a preceding fast), a 24-hour sample, or a fasted overnight sample.

The level of MI in the sample can be measured by any of the analytical procedures known to those skilled in the art. Illustrative examples of suitable analytical procedures include, but are not limited to, high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), immunoassays, enzyme assays, and gas chromatography/mass spectroscopy (GC/MS) (*see, e.g.*,

U.S. Patent No. 5,525,526; U.S. Patent No. 5,356,750; *Anal. Biochem.*, *158*:59-63 (1986); Olsson *et al.*, *Anal. Chim. Acta*, *206*:49-55 (1988); MacGregor *et al.*, *Anal. Biochem.*, *141*:382-389 (1984); and Inamoto *et al.*, *Proc. Japan Clin. Chem. Ann.*, *28*:116 (1988)). Preferably, the level of MI in the sample is measured by an enzyme assay.

Preferably, when the bodily fluid is urine, the level of MI in the sample is determined as: (i) concentration of MI (nmol/mL); or (ii) total daily excretion of MI (μmol/day). Increased levels of MI in urine indicate a loss of glycemic control, while decreased levels of MI indicate improvement of glycemic control.

As an illustrative example, the lower reference level for a spot urine sample is in the range of 10 to 20 nmol/mL. MI levels below this lower reference level indicate good glycemic control. It should be pointed out, however, that these exemplary levels are from one specific analytical procedure and may differ depending on the particular analytical procedure employed.

As another illustrative example, the upper reference level for a spot urine sample is in the range of 20-45 nmol/mL. MI levels above this upper reference level indicate poor glycemic control. It should be pointed out, however, that these exemplary levels are from one specific analytical procedure and may differ depending on the particular analytical procedure employed.

The predicative value of MI levels in assessing glycemic control correlates well with the predictive value of both fructosamine levels and glycated hemoglobin levels.

The following example is illustrative only and is not intended to limit the scope of the invention as defined by the appended claims. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods of the present invention without departing from the spirit and scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

All patents and publications referred to herein are expressly incorporated by reference.

EXAMPLE I: A STUDY OF URINARY MYO-INOSITOL LEVELS IN PATIENTS WITH TYPE II DIABETES

A study was conducted in one-hundred and twenty-eight (128) patients clinically diagnosed with Type II diabetes. The group consisted of forty-seven (47) males and eighty-one (81) females.

Immediately prior to collecting a fasting blood sample, a spot urine sample was collected from each subject. MI levels were measured in the urine samples by a gas chromatography/mass spectrometer procedure. Glucose, fructosamine, and glycated hemoglobin levels were measured in the fasting blood sample.

There was a highly significant correlation ($P<0.0001$) between the urinary MI level and the fasted blood glucose, fructosamine, and glycated hemoglobin levels (FIGS 1-3).

WHAT IS CLAIMED IS:

1. A method for assessing the degree of glycemic control in a mammal, said method comprising:

(a) obtaining a sample of bodily fluid from said mammal;

(b) measuring the amount of *myo*-inositol in said sample to obtain a sample *myo*-inositol level;

(c) comparing said sample *myo*-inositol level to a previously defined *myo*-inositol reference level indicative of glycemic control; and

(d) assessing the degree of glycemic control in said mammal.

2. The method of claim 1, wherein said bodily fluid is urine.

3. The method of claim 1, wherein said bodily fluid is blood.

4. The method of claim 1, wherein said mammal is a human.

5. The method of claim 1, wherein a sample *myo*-inositol level above said previously defined *myo*-inositol reference level indicates poor glycemic control.

6. The method of claim 1, wherein a sample *myo*-inositol level below said previously defined *myo*-inositol reference level indicates good glycemic control.

7. The method of claim 2, wherein said previously defined *myo*-inositol reference

level is in the range of 20 to 45 nmol/mL.

8. The method of claim 2, wherein said previously defined *myo*-inositol reference level is in the range of 10 to 20 nmol/mL.

9. A method for diagnosing the degree of insulin resistance in a mammal, said method comprising:

(a) obtaining a sample of bodily fluid from said mammal;

(b) measuring the amount of *myo*-inositol in said sample to obtain a sample *myo*-inositol level;

(c) comparing said sample *myo*-inositol level with at least one previously defined *myo*-inositol reference level; and

(d) diagnosing the degree of insulin resistance in said mammal.

10. The method of claim 9, wherein said bodily fluid is urine.

11. The method of claim 9, wherein said bodily fluid is blood.

12. The method of claim 9, wherein said mammal is a human.

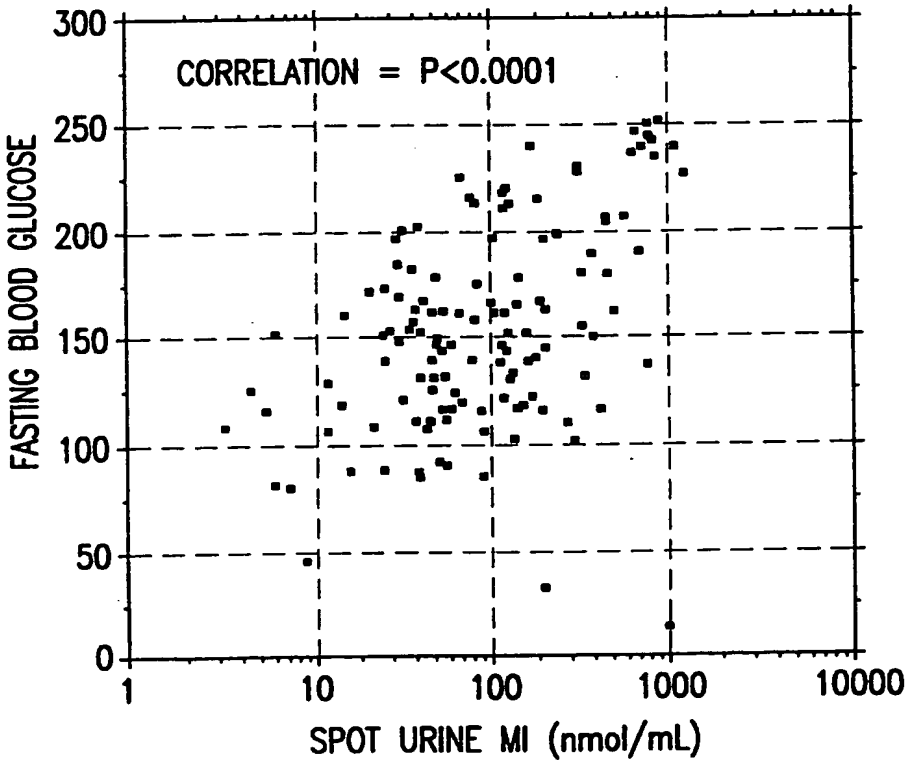

CORRELATION = P<0.0001
FASTING BLOOD GLUCOSE
SPOT URINE MI (nmol/mL)
300
250
200
150
100
50
0
1
10
100
1000
10000

FIG.1

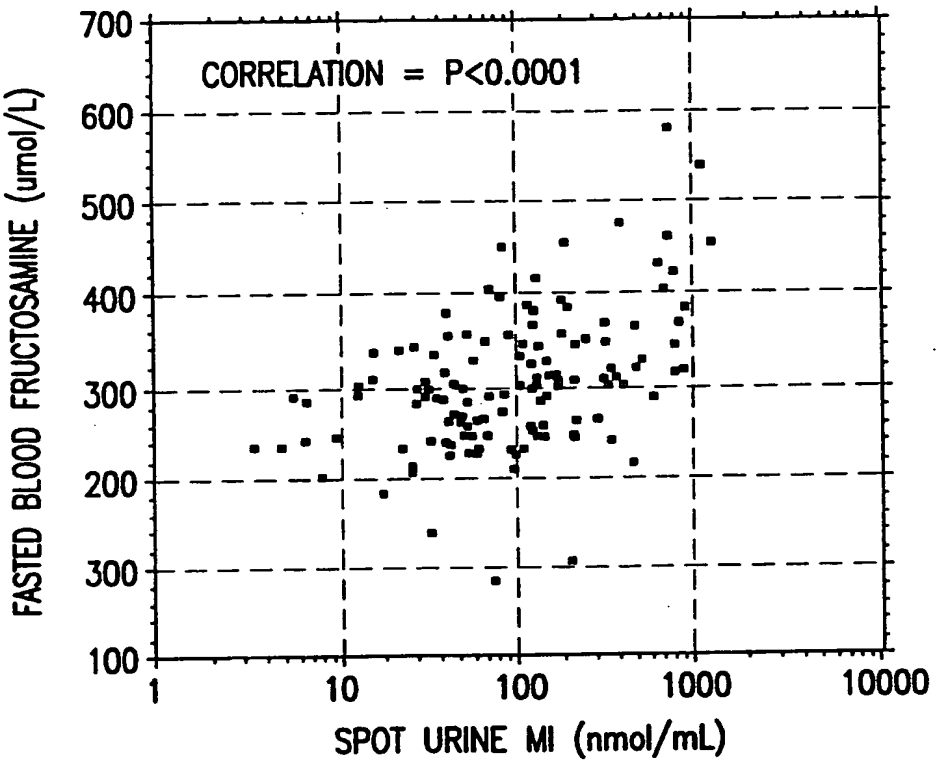

CORRELATION = P<0.0001
FASTED BLOOD FRUCTOSAMINE (umol/L)
700
600
500
400
300
200
300
100
1
10
100
1000
10000
SPOT URINE MI (nmol/mL)

FIG.2

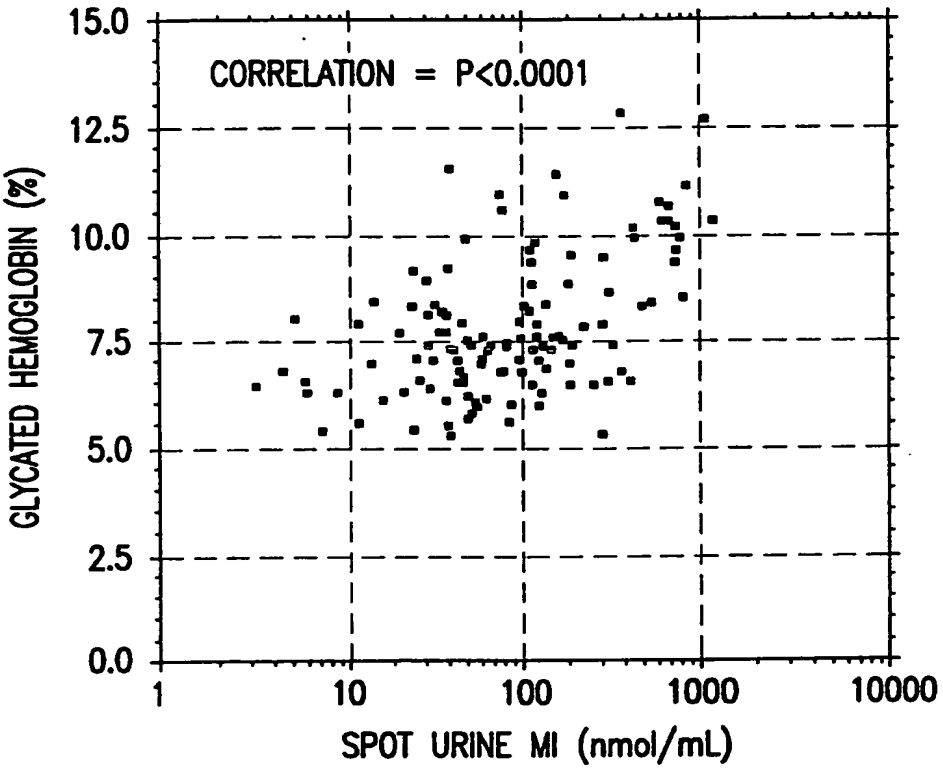

CORRELATION = P<0.0001
GLYCATED HEMOGLOBIN (%)
15.0
12.5
10.0
7.5
5.0
2.5
0.0
1
10
100
1000
10000
SPOT URINE MI (nmol/mL)

FIG.3

INTERNATIONAL SEARCH REPORT

Interne onal Application No
PCT/US 99/11201

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/66

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R. DOLHOFER ET AL.: "Enzymatic assay of myo-inositol in serum." JOURNAL OF CLINICAL CHEMISTRY AND CLINICAL BIOCHEMISTRY, vol. 25, no. 10, 1987, pages 733-736, XP002114799 Berlin, DE the whole document ---	1-12
X	E. PITKÄNEN: "The serum polyol pattern and the urinary polyol excretion in diebetic and in uremic patients." CLINICA CHIMICA ACTA, vol. 38, 1972, pages 221-230, XP002114800 Amsterdam, NL. cited in the application page 225 -page 228 --- -/--	1-12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 September 1999	22/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Griffith, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter onal Application No
PCT/US 99/11201

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9601996 A	25-01-1996	US 5750348 A	12-05-1998
		CA 2194667 A	25-01-1996
		EP 0770213 A	02-05-1997
		JP 10507826 T	28-07-1998